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<p>(54) Title: METHOD TO INHIBIT RESTENOSIS</p> <p>(57) Abstract</p> <p>A pharmaceutical composition to inhibit the development of intimal hyperplasia following vascular intervention procedures (angioplasty) is disclosed. The pharmaceutical composition contains green porphyrin and is administered to the subject concurrent with and following the angioplasty. No purposeful irradiation with light absorbed by the green porphyrin is required or employed with the composition.</p>		

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5                    METHOD TO INHIBIT RESTENOSISTechnical Field

                  The invention concerns pharmaceutical  
compositions for preventing development of restenosis  
10 or intimal hyperplasia. This process is a commonly  
occurring side effect of angioplasty. More  
specifically, the invention concerns inhibition of  
restenosis by administering a pharmaceutical  
composition containing green porphyrin to the  
15 angioplasty subject roughly concurrently with the  
angioplasty procedure and for several months following  
the procedure. No purposeful irradiation with light  
is needed to effect the desired inhibition.

20                    Background Art

                  Invasive manipulation of the peripheral  
circulatory system to correct occlusive diseases of  
the arteries has become more and more routine. Over  
200,000 procedures are performed annually in the  
25 United States alone which involve blood vessel  
bypasses, balloon catheters, and other "mechanical"  
techniques to correct the problem. A serious side  
effect of these procedures is the subsequent  
development in the subject of intimal hyperplasia  
30 which may, itself, constitute a blockage problem.  
This appears to be a direct response to the intimal  
injury caused by the intervention; smooth muscle cells  
and fibroblasts proliferate and create stenoses in the  
interior of the vascular wall. The mechanism of this  
35 process is evidently not well understood, but a  
central feature of the development of the problem

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5 appears to be unwanted proliferation of smooth muscle cells.

Various methods have been tried to overcome this dangerous side effect. Approaches have included mechanical manipulation as well as administration of chemical agents such as aspirin, dexamethasone, 10 heparins, calcium channel blockers, and a variety of other agents presumed on the basis of various theories to interfere with the development of the stenoses. They have met with very little success and have side effects of their own.

15 One additional approach is the use of photodynamic therapy (PDT). This form of management, originally applied to cancer treatment, involves the use of photoactive materials which home to tumor tissue, presumably because of the rapidly 20 proliferating nature of the tissue. The photoactive substances, which include psoralen, various porphyrin-based materials, such as Photofrin II™ porphyrin aggregate, chlorins, phthalocyanines, and monohydrobenzoporphyrin derivatives, to name but a 25 few, are harmless unless photoactivated. However, when irradiated with light of appropriate wavelength, the drugs apparently effect the formation of a toxic agent, presumably singlet oxygen, although they themselves are chemically unchanged. The resultant 30 toxic agent causes the destruction of the unwanted tumor tissue.

PDT has also been applied with some success to the treatment of atherosclerotic plaques. See, for example, Kessel, D. et al., Photochem Photobiol (1984) 35 40:59-62; Okunaka, T. et al., Photochem Photobiol (1987) 46:769-775; Spears, J.R. et al., J Clin Invest (1983) 71:395-399; Spokqiny, A.M. et al., J Am Col Cardiol (1986) 8:1387-1392; Copperath, K. et al., Eur

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Heart J (1989) 10 (Suppl):151; Straight, R. et al.,  
5 Photodynamic Therapy of Tumors and Other Diseases,  
(1985) pp. 349-350.

The use of PDT to treat or prevent the  
restenosis that often accompanies angioplasty has also  
been studied. These studies have either employed  
10 smooth muscle cells in (SMC) in culture, on the theory  
that SMC proliferation is the *sine qua non* of  
restenosis, or have used animal models. In general,  
PDT appears to show promise in this regard. However,  
controls run in many of these studies, using the  
15 photoactivating agent in the absence of light, have  
provided contradictory results when smooth muscle  
cells in culture were used as the model system.  
Applicants are unaware of any animal studies which  
showed any indication of positive results for  
20 preventing restenosis in the absence of light.

Dartsch, P.C. et al. reported in Advances in  
Laser Medicine 4: Laser Angioplasty II Biamino, G., et  
al. (eds.) Ecomed Verlagsgesellschaft, Landsberg/Lech,  
Berlin (1990) 77-80, the results of contracting smooth  
25 muscle cells in culture exposed to dihematoporphyrin-  
ester and -ether (DHE). This porphyrin-based drug is  
now marketed as Photofrin II". The report discloses  
that SMC were isolated by enzymatic disaggregation of  
either normal or stenosing plaque tissues and cultured  
30 in vitro. They were tested in their first, second or  
third passage by treating them with DHE at  
concentrations ranging from 0.1-25 µg/ml and  
irradiated with ultraviolet light. The percentage of  
viable and still adherent cells was markedly reduced  
35 for plaque-derived SMC and much less dramatically  
reduced for normal SMC. A less dramatic, but  
nevertheless detectable, effect was observed in the  
presence of DHE but in the absence of radiation.

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Specifically, cells treated with 5  $\mu\text{g/ml}$  DHE and light  
5 showed a reduction in the number of viable cells to  
73% in the case of normal derived cells and 38% for  
plaque-derived SMC. Using a DHE concentration of 1  
 $\mu\text{g/ml}$  and an energy density of 1200  $\text{mJ/cm}^2$ , after 24  
10 hours 80% of the normal SMC and 20% of the plaque-  
derived SMC were viable. Similar results were  
reported by this group in related publications:  
Dartsch, P.C. et al., Atherosclerosis (1990) 10:616-  
624; Dartsch, P.C. et al., J Am Coll Cardiol (1990)  
15:1545-1550.

15 In two recent reports by Sobeh, M.S. et al.,  
results apparently contradictory to those of Dartsch,  
et al. were obtained when SMC cultured from the  
intermedia of human long saphenous vein harvested for  
coronary artery vein bypass grafting, were used in the  
20 tests. These cells were treated with Photofrin II<sup>™</sup>  
porphyrin aggregates and irradiated. These reports  
state that the cells were unaffected by Photofrin II<sup>™</sup>  
porphyrin aggregate at 0-100  $\mu\text{g/ml}$  without light.  
However, when treated with light energy of greater  
25 than 3  $\text{J/cm}^2$  in the presence at least 2  $\mu\text{g/ml}$  of the  
drug, a mean cell destruction of over 80% was reported  
regardless of wavelength. The reports also state that  
light without prior chromophore sensitization produced  
no cell damage. (Vascular Surgical Society of Great  
30 Britain and Ireland Annual General Meeting, London,  
November 1992; 13th Annual American Society of Laser  
Medicine and Surgery, New Orleans, April 1993.)

Asahara, T. et al. reported in Circulation  
(1992) 86 (Suppl) I-846, that PDT was able to inhibit  
35 restenosis in rabbits that had received balloon  
injuries of the iliac artery and were fed with a 0.2%  
cholesterol diet. Hematoporphyrin derivative (HPD)  
was administered 24 hours before irradiation at

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various times relative to the injury. The best  
5 effects were observed when the treatment was  
administered one week after angioplasty.

In another *in vivo* study, Ortu, P. et al.,  
Circulation (1992) 85:1189-1196, reported that  
10 photodynamic therapy, with chloraluminum-sulfonated  
phthalocyanine (CASPc) used as the drug, was effective  
in inhibiting the intimal hyperplasia response in rats  
subjected to balloon injury of the carotid artery.  
Controls consisted of rats irradiated, but not  
administered the drug. No controls using CASPc  
15 without light were reported.

Eton, D. et al., J. Surg. Res. (1992)  
53:558-562, reported the effect of photodynamic  
therapy using Photofrin II™ porphyrin aggregates in a  
20 rabbit model wherein the rabbits underwent  
standardized intimal injury to both common carotid  
arteries with a balloon catheter. The test animals  
received Photofrin II™ porphyrin aggregate and  
subsequent irradiation; the control groups either  
received no treatment, or chromophore alone, or light  
25 alone. The results were evaluated in terms of  
arterial cross sections. Only the test group showed a  
statistically significant improvement over the  
controls, although the animals treated with light  
alone or Photofrin II™ porphyrin aggregate alone had  
30 non-significant lower mean ratios of the area of  
intimal hyperplasia to the area enclosed by the  
internal elastic lamina, used as a measure of stenosis  
in this study.

It would be advantageous to provide a  
35 treatment to prevent intimal hyperplasia (IH)  
following vascular trauma which is independent of PDT,  
so that the necessity to provide light using  
specialized equipment is avoided. It has now been

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found that green porphyrins, administered concurrently  
5 with, and for a period of time after, angioplastic  
procedures, can effectively inhibit the undesired  
stenoses often accompanying this procedure.

#### Disclosure of the Invention

10 The invention provides pharmaceutical  
methods for preventing or inhibiting the intimal  
hyperplasia and resultant restenosis which occur as  
side effects of corrective vascular treatments. The  
pharmaceutical composition is administered to a  
15 subject undergoing an angioplasty, concurrently with  
this procedure, and contains an amount of green  
porphyrin effective to interfere with the development  
of restenosis. The administration of the drug need  
not be accompanied by purposeful irradiation with  
20 light; indeed, the treatment is performed without  
irradiation by light absorbed by the green porphyrin  
administered.

Thus, in summary, the invention is directed  
to a pharmaceutical composition for inhibiting the  
25 development of intimal hyperplasia following  
angioplasty. The composition is administered to a  
subject, in conjunction with the angioplasty, and  
contains an amount of green porphyrin (Gp) effective  
to inhibit said development. The composition allows  
30 inhibition to occur in the absence of purposeful  
irradiation with light absorbed by the Gp.

#### Brief Description of the Drawings

Figure 1 shows the formulas of typical green  
35 porphyrins useful in the invention method.

Figure 2 shows the formulas of 4  
particularly preferred embodiments of the green



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porphyrins of the invention, BPD-DA, BPD-DB, BPD-MA,  
5 and BPD-MB.

#### Modes of Carrying Out the Invention

The composition of the invention is intended to prevent the undesired side effects of angioplasty.  
10 As used herein, "angioplasty" refers to surgical procedures which traumatize the vascular walls. Such procedures include, but are not limited to, femoral-popliteal bypasses, femoral-tibial bypasses, aorto-iliac bypasses, coronary bypasses, percutaneous  
15 transluminal angioplasty, balloon angioplasty, laser angioplasty and directional atherectomy. Any procedure which involves traumatic manipulation of the vasculature is included in this definition.

As used herein, intimal hyperplasia (IH) is  
20 defined as a pathophysiological phenomenon which results in the occlusion of the vasculature and is accompanied by the proliferation of cells including smooth muscle cells at the interior of the blood vessels. It is not implied by this definition that  
25 the method of the invention necessarily directly inhibits the proliferation of SMC; however, the condition which the method of the invention is designed to inhibit includes such proliferation.

By "in conjunction with angioplasty" is  
30 meant application of the method beginning at a time roughly accompanying the angioplastic procedure. This may include multiple or single treatments in the ensuing days, weeks or months after the procedure is performed. "In conjunction with" refers to a time  
35 period within the ambit of the effects of the angioplasty procedure. Typically, an initial dose of green porphyrin will be within 6-12 hours of the angioplasty, preferably within 6 hours thereafter.

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Follow-up dosages may then be made at weekly,  
5 biweekly, or monthly intervals. Design of particular  
protocols depends, of course, on the individual  
subject, the condition of the subject, the design of  
the dosage levels, and the judgment of the attending  
practitioner. Preferably, the green porphyrin is  
10 administered in the form of 2-5 doses, the first dose  
being administered within six hours of angioplasty.

The green porphyrins useful in the method of  
the invention are described in detail in the issued  
U.S. patent 5,171,749 which is incorporated herein in  
15 its entirety by reference. These compounds are  
porphyrin derivatives obtained by reacting a porphyrin  
nucleus with an alkyne in a Diels-Alder type reaction  
to obtain a monohydrobenzoporphyrin. Preferred  
embodiments of the Gp are those wherein the resulting  
20 Diels-Alder product is rearranged and partially  
hydrolyzed. A particularly preferred set of  
embodiments is designated in the referenced patent as  
BPD-DA, -DB, -MA, and -MB. Particularly preferred and  
exemplified herein is BPD-MA.

25

#### The Green Porphyrins

In further detail with respect to the green  
porphyrins useful in the invention, the general  
structures of typical green porphyrins are formulas  
30 1-6 as shown in Figure 1. Particularly preferred  
forms are shown in Figure 2.

Gp is selected from a group of porphyrin  
derivatives obtained using Diels-Alder reactions of  
acetylene derivatives with protoporphyrin under  
35 conditions which effect a reaction at only one of the  
two available conjugated, non-aromatic diene  
structures present in the protoporphyrin-IX ring  
system (rings A and B). The formulas shown in Figure

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1 represent typical green porphyrins useful in the  
5 invention. These compounds are shown in the figure  
with hydrogen occupying the internal ring nitrogens;  
however, it is understood that the metalated forms  
wherein a cation replaces one or both of these  
hydrogens can also be employed. It is also understood  
10 that these compounds can be labeled either by  
replacement of one or more of the atoms in the  
structure by its radioactive form, or by coupling to a  
radioisotope such as a radioactive metal or, for  
example, a radioisotope of iodine.

15 For convenience, an abbreviation of the term  
hydro-monobenzoporphyrin derivative -- "BPD" -- is  
generally used to refer to compounds of formulas 3 and  
4 of Figure 1. These are the preferred forms of Gp.  
As shown in Figure 1, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are  
20 non-interfering substituents which do not affect,  
appreciably, the activity of the compound in the  
invention method. Most typically, R<sup>1</sup> and R<sup>2</sup> are  
carbalkoxy groups, typically methyl or ethyl carboxy  
esters. Most commonly R<sup>3</sup> is 2-carboxyethyl or the  
25 alkyl ester thereof and R<sup>4</sup> is vinyl. These preferred  
embodiments result from the availability of native  
porphyrins and are non mandated by considerations of  
biological efficacy. By "non-interfering  
substituents" is meant substituents which do not  
30 destroy the ability of the green porphyrin to inhibit  
hyperplasia.

Dimeric forms of the Gp and dimeric and  
multimeric forms of Gp/porphyrin combinations can also  
be employed.

35 "Green porphyrin" refers to a porphyrin  
nucleus modified, for example, by a Diels-Alder  
reaction involving the conjugated system comprising a  
pyrrole nucleus and a vinyl substituent with an

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acetylene dienophile. This results in a fused  
5 cyclohexadiene -- referred to herein as  
"hydrobenzo" -- fused to the A or B ring, as shown in  
formulas 1 and 2. Rearrangement of the  $\pi$  system in  
the hexadiene ring results in the compounds of  
formulas 3 and 4; reduction provides the compounds of  
10 formulas 5 and 6. Specific preparation of this class  
of Gp compounds useful in the invention is described  
in detail in U.S. patent 5,095,030 incorporated herein  
by reference.

For the compounds shown in Figures 1 and 2,  
15 generally,  $R^1$  and  $R^2$  are each, independently, moderate  
electron- withdrawing substituents, and are, most  
commonly, carbalkoxy, alkyl or aryl sulfonyl, or any  
other activating substituents which are not  
sufficiently electron-withdrawing to result in  
20 reaction with both A and B rings rather than reaction  
with only one. One of  $R^1$  and  $R^2$  may optionally be H  
while the other is an electron withdrawing substituent  
of sufficient strength to facilitate the Diels-Alder  
reaction.

As used herein, carboxy is, as  
25 conventionally defined, -COOH and carbalkoxy is -COOR,  
wherein R is alkyl; carboxyalkyl refers to the  
substituent -R'-COOH wherein R' is alkylene;  
carbalkoxyalkyl refers to -R'-COOR wherein R' and R  
30 are alkylene and alkyl respectively. Alkyl is a  
saturated straight or branched chain hydrocarbyl of  
1-6 carbon atoms such as methyl, n-hexyl,  
2-methylpentyl, t-butyl, n-propyl, and so forth.  
Alkylene is as alkyl except that the group is  
35 divalent. Aryl or alkyl sulfonyl moieties have the  
formula  $SO_2R$  wherein R is alkyl as above-defined, or is  
aryl, wherein aryl is phenyl optionally substituted  
with 1-3 substituents independently selected from halo

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5 (fluoro, chloro, bromo or iodo), lower alkyl (1-4C) or lower alkoxy (1-4C). In addition, one or both  $R^1$  of  $R^2$  can itself be aryl -- i.e., phenyl optionally substituted as above defined.

In a preferred embodiment,  $R^1$  and  $R^2$  are carbomethoxy and carboethoxy.

10 As shown in Figure 1, the adduct formed by the reaction of  $R^1-C\equiv C-R^2$  with the protoporphyrin-IX ring system ( $R^3$  is a protected form of 2-carboxyethyl such as 2-carbomethoxyethyl or 2-carboethoxyethyl;  $R^4$  is  $CH=CH_2$ ) are compounds of the formulas 1 and 2  
15 wherein the compound in formula 1 results from addition to the A ring and formula 2 results from addition to the B ring. In these resulting products of formulas 1 and 2,  $R^4$  remains  $CH=CH_2$ , however this vinyl group is readily derivatized to other  
20 embodiments of  $R^4$  by addition to or oxidation of the vinyl ring substituent of ring B in formula 1 or ring A in formula 2. The addition or oxidation products can be further substituted if the added substituents are functional leaving groups -- for example -Br may  
25 be substituted by -OH, -OR (R is alkyl 1-6C as above), or -NH<sub>2</sub>, -NHR, -NR<sub>2</sub>, etc. In preferred embodiments, one of the added substituents is hydrogen, and the other is selected from the group consisting of halo (fluoro, chloro, bromo or iodo), hydroxy, lower  
30 alkoxy, amino or an amide, sulfhydryl or an organo-sulfide or can be, itself, hydrogen. The product of the Markovnikov addition of water provides a substituent structure analogous to the hematoporphyrin ring system at the relevant ring. Thus, the compounds  
35 of the invention include various groups as  $R^4$ , including substituents which provide additional

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5 porphyrin or porphyrin-related ring systems, as will  
be further described below.

10  $R^3$  in protoporphyrin-IX is 2-carboxyethyl  
( $-\text{CH}_2\text{CH}_2\text{COOH}$ ). However, the nature of  $R^3$  (unless it  
contains a  $\pi$ -bond conjugated to ring  $\pi$ -bond), is  
ordinarily not relevant to the progress of the Diels-  
Alder reaction or to the effectiveness of the  
15 resulting product.  $R^3$  can thus be, for example, lower  
alkyl (1-4C), or  $\omega$ -carboxyalkyl (2-6C) or the esters  
or amides thereof. The  $R^3$  substituent may also be  
substituted with halogen as above-defined, or with  
20 other nonreactive substituents. However, as the  
convenient starting materials for the Gp compounds of  
the invention are the naturally occurring porphyrins,  
the preferred substituents for  $R^3$  are  $-\text{CH}_2\text{CH}_2\text{COOH}$  or  
 $-\text{CH}_2\text{CHR}_2\text{COOR}$ , wherein R is alkyl (1-6C).

25 The hydro-monobenzoporphyrins which directly  
result from the Diels-Alder reaction described in the  
cited references can also be isomerized to compounds  
of formulas shown as 3 and 4 of Figure 1. The  
depictions of compounds 3 and 4 in Figure 1 do not  
show the relative position of the exocyclic methyl  
group (ring A of formula 3 and ring B of formula 4)  
with respect to the  $R^2$  substituent. Either isomer is  
available.

30 In addition, the Diels-Alder products can be  
selectively reduced by treating with hydrogen in the  
presence of palladium on charcoal to give the  
saturated ring analogs, shown as formulas 5 and 6 in  
Figure 1, corresponding to the respective Diels-Alder  
products of rings A and B. These reduced products are  
35 less preferred embodiments, and are less useful in the  
method of the invention than the compounds of formulas  
1-4.

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5           The description set forth above with respect  
to the compounds of formulas 1 and 2 concerning  
derivatization by conversion of the remaining vinyl  
substituent ( $R^4$ ) and with respect to variability of  $-R^3$   
applies as well to the compounds of formulas 3, 4, 5  
and 6.

10           In the BPD compounds of the invention, it  
has been found advantageous to hydrolyze or partially  
hydrolyze the esterified carboxy group in  $-\text{CH}_2\text{CH}_2\text{COOR}$ .  
The hydrolysis occurs at a much faster rate than that  
of the ester groups of  $R^1$ ,  $R^2$ , and the solubility and  
15       biodistribution characteristics of the resulting  
compounds are more desirable than those of the  
unhydrolyzed form. Hydrolysis results in the di-acid  
or monoacid products (or their salts).

20           The compounds of formulas 3 and 4 (BPD), and  
especially those which have hydrolyzed and partially  
hydrolyzed carbalkoxy groups in  $R^3$ , are most preferred.  
Compounds of the invention which contain  $-\text{COOH}$  may be  
prepared as the free acid or in the form of salts with  
organic or inorganic bases.

25           Figure 2 shows four particularly preferred  
compounds of the invention. These compounds are  
collectively designated benzoporphyrin derivative  
(BPD) as they are forms of Gp having the formula 3 or  
4. These are hydrolyzed or partially hydrolyzed forms  
30       of the rearranged products of formula 3 and 4, wherein  
one or both of the protected carboxyl groups of  $R^3$  are  
hydrolyzed. The ester groups at  $R^1$  and  $R^2$  hydrolyze  
relatively so slowly that conversion to the forms  
shown in Figure 2 is easily effected.

35           For purposes of this description,  $R^3$  is  
 $-\text{CH}_2\text{CH}_2\text{COOR}^3$ . As shown in Figure 2, each  $R^3$  is H in  
preferred compound BPD-DA,  $R^1$  and  $R^2$  are carbalkoxy,

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and derivatization is at ring A; BPD-DB is the  
5 corresponding compound wherein derivatization is at  
ring B. BPD-MA represents the partially hydrolyzed  
form of BPD-DA, and BPD-MB, the partially hydrolyzed  
form of BPD-DB. Thus, in these latter compounds, R<sup>1</sup>  
and R<sup>2</sup> are carbalkoxy, one R<sup>3</sup> is H and the other R<sup>3</sup> is  
10 alkyl (1-6C). The compounds of formulas BPD-MA and  
BPD-MB may be homogeneous wherein only the C ring  
carbalkoxyethyl or only the D ring carbalkoxyethyl is  
hydrolyzed, or may be mixtures of the C and D ring  
substituent hydrolyzates. In addition, mixtures of  
15 any two or more of BPD-MA, -MB, -DA and -DB may be  
employed in the method of the invention.

It will be noted that many of the compounds  
of Figure 1 contain at least one chiral center and  
therefore exist as optical isomers. The method of the  
20 invention can employ compounds having both configura-  
tions of the chiral carbons, whether the compounds  
are supplied as isolates of a single stereoisomer or  
are mixtures of enantiomers and/or diastereomers.  
Separation of mixtures of diastereomers may be  
25 effected by any conventional means; mixtures of  
enantiomers may be separated by usual techniques of  
reacting them with optically active preparations and  
separating the resulting diastereomers.

It should further be noted that the reaction  
30 products may be unseparated mixtures of A and B ring  
additions, e.g., mixtures of formulas 1 and 2 or 3 and  
4 or 5 and 6. Either the separated forms -- i.e.,  
formula 3 alone or 4 alone, or mixtures in any ratio  
may be employed in the methods of therapy and  
35 diagnosis set forth herein.

Generally, and in summary with respect to  
substituents, each R<sup>1</sup> and R<sup>2</sup> is independently selected  
from the group consisting of carbalkoxy (2-6C), alkyl



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(1-6C) sulfonyl, aryl (6-10C) sulfonyl, aryl (6-10C);  
 5 cyano; and  $-\text{CONR}^5\text{CO}-$  wherein  $\text{R}^5$  is aryl (6-10C) or  
 alkyl (1-6C);

each  $\text{R}^3$  is independently  $\omega$ -carboxyalkyl (2-  
 6C) or a salt, amide, ester or acylhydrazone thereof,  
 or is alkyl (1-6C); and

10  $\text{R}^4$  is vinyl,  $\text{CHCH}_2$ ,  $\text{CHOR}^{4'}$ ,  $-\text{CHO}$ ,  $-\text{COOR}^{4'}$ ,  
 $\text{CH}(\text{OR}^{4'})\text{CH}_3$ ,  $\text{CH}(\text{OR}^{4'})\text{CH}_2\text{OR}^{4'}$ ,  $-\text{CH}(\text{SR}^{4'})\text{CH}_3$ ,  $-\text{CH}(\text{NR}^{4'})_2\text{CH}_3$ ,  
 $-\text{CH}(\text{CN})\text{CH}_3$ ,  $-\text{CH}(\text{COOR}^{4'})\text{CH}_3$ ,  $-\text{CH}((\text{OOCR}^{4'})\text{CH}_3$ ,  $-\text{CH}(\text{halo})\text{CH}_3$ ,  
 or  $-\text{CH}(\text{halo})\text{CH}_2(\text{halo})$ ,

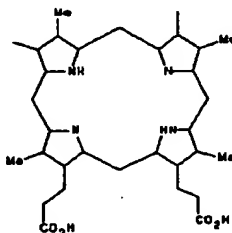
wherein  $\text{R}^{4'}$  is H, alkyl (1-6C) optionally  
 15 substituted with a hydrophilic substituent, or

wherein  $\text{R}^4$  is an organic group of  $<12\text{C}$   
 resulting from direct or indirect derivatization of  
 vinyl, or

wherein  $\text{R}^4$  is a group containing 1-3  
 20 tetrapyrrole-type nuclei of the formula  $-\text{L}-\text{P}$  as herein  
 defined.

Compounds of the formulas 3 and 4 and  
 mixtures thereof are particularly preferred. Also  
 preferred are those wherein  $\text{R}^1$  and  $\text{R}^2$  are the same and  
 25 are carbalkoxy, especially carboethoxy; also preferred  
 are those wherein  $\text{R}^4$  is  $-\text{CHCH}_2$ ,  $\text{CH}(\text{OH})\text{CH}_3$  or  $-\text{CH}(\text{halo})$   
 $\text{CH}_3$ , or is a group containing 1-3 tetrapyrrole-type  
 nuclei of the formula  $-\text{L}-\text{P}$  (defined below).

As used herein, "tetrapyrrole-type nucleus"  
 30 represents a four-ring system of the skeleton:



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and a salt, ester, amide or acylhydrazone thereof,  
5 which is highly conjugated. It includes the porphyrin  
system, which is, in effect, a completely conjugated  
system, the chlorin system, which is, in effect, a  
dihydro form of the porphyrin, and the reduced chlorin  
10 system, which is a tetrahydro form of the completely  
conjugated system. When "porphyrin" is specified, the  
completely conjugated system is indicated; Gp is  
effectively a dihydro form of the porphyrin system.

One group of compounds is that wherein the  
substituent R<sup>4</sup> includes at least one additional  
15 tetrapyrrole-type nucleus. The resulting compounds of  
the invention are dimers or oligomers in which at  
least one of the tetrapyrrole-type ring systems is Gp.  
Linkage between the Gp moiety through the position of  
R<sup>4</sup> to an additional tetrapyrrole-type ring system may  
20 be through an ether, amine or vinyl linkage.  
Additional derivatization in the case of porphyrin  
ring systems which have two available substituent  
positions (in both A and B rings) corresponding to R<sup>4</sup>  
can also be formed, as further described below.

25 As stated above, the compounds of formulas  
shown in Figure 1 include those wherein the embodiment  
of R<sup>4</sup> is formed by addition to the vinyl groups of  
initial Gp products. Thus, R<sup>4</sup> can be any substituent  
consistent with that formed by a facile addition  
30 reaction. Thus, both added substituents can be, for  
example, OH or halo, and these substituents can be  
further substituted, or the addition reagent may be of  
the form HX wherein H is added to the ring-adjacent  
carbon to provide R<sup>4</sup> of the form

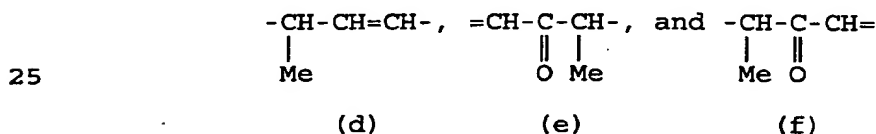
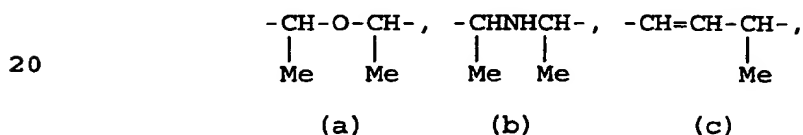


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The vinyl group can also be oxidized to  
 5 obtain  $R^4$  as  $CH_2OH$ ,  $-CHO$ , or  $COOH$  and its salts and  
 esters.

Thus, in general  $R^4$  represents any  
 substituents to which the vinyl group  $-CH=CH_2$  is  
 readily converted by cleavage or addition, and further  
 10 resultants of reaction of leaving groups with  
 additional moieties. Typical  $R^4$  substituents include:  
 $CH(OH)Me$ ,  $-CHBrMe$ ,  $-CH(OMe)Me$ ,  $-CH(\text{pyridinium}$   
 $\text{bromide})Me$ ,  $-CH(SH)Me$  and the disulfide thereof,  
 $-CHOHCH_2OH$ ,  $-CHO$ , and  $-COOH$  or  $-COOMe$ .

15 When  $R^4$  is  $-L-P$ , the substituent formula  
 $"-L-P"$  represents a substituent wherein  $-L-$  is  
 selected the group consisting of



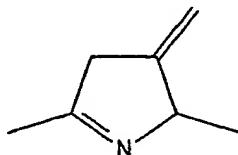
and  $P$  is selected from the group consisting of  $Gp$   
 wherein  $Gp$  is of the formula 1-6 shown in Figure 1,  
 30 but lacking  $R^4$  and conjugated through the position  
 shown in Figure 5 as occupied by  $R^4$  to  $L$ , and a  
 porphyrin.

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(It is also understood that when -L- is of  
5 the formula (e) or (f), the ring system to which the  
double bond is attached will have a resonance system  
corresponding to

10



in the ring to which the double bond is attached, as  
shown.)

15 The dimers and oligomeric compounds of the  
invention can be prepared using reactions analogous to  
those for dimerization and oligomerization of  
porphyrins per se. The green porphyrins or green  
porphyrin/ porphyrin linkages can be made directly, or  
20 porphyrins may be coupled, followed by a Diels-Alder  
reaction of either or both terminal porphyrins to  
convert to the corresponding green porphyrin.

#### Detailed Description of the Method

25 In the method of the invention, the subject  
is administered an amount of the Gp compound or a  
mixture of Gp compounds in one or several dosages.  
Suitable amounts for total dosage are in the range of  
0.04-20 mg/kg body weight; preferably 0.2-8 mg/kg body  
30 weight. Typical amounts per dose are in the range of  
0.01-5 mg/kg or preferably 0.05-2 mg/kg. These dosage  
ranges are intended to be suggestive and not limiting,  
since of course the individual reactions of particular  
subjects will vary. Adjustment of the dosage ranges  
35 in accordance with these variations is routine among  
practitioners.

Similarly, no single protocol is desirable  
for all cases. However, typical protocols will

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5 include an initial dose administered within six hours before or after the angioplasty procedure followed by 1-4 additional doses at weekly, biweekly or monthly intervals. Again, these protocols are not intended to be limiting in view of the wide variation in protocol design permitted.

10 The Gp of the invention may be administered as a single compound, preferably BPD-MA, or as mixtures of various Gp's. Suitable formulations include those appropriate for systemic administration, including preparations for injection, transmucosal or  
15 transdermal administration, or even oral administration. A particularly preferred means of formulating the Gp of the invention for this use is in the form of liposomes or a liposomal formulation. The Gp may be included within the liposomes, attached to  
20 their surface, or both. Suitable methods for preparing liposomes are well known in the art, and inclusion of Gp in such preparations is described in U.S. patent 5,214,036 and U.S. patent application 07/832,542 filed 15 March 1993, both of which are  
25 incorporated herein by reference. As stated above, the Gp compounds and formulations are administered without the necessity of irradiating the site of potential restenosis with light absorbed by the Gp. By "in the absence of irradiation with light absorbed  
30 by said Gp" is meant that no such deliberate irradiation is administered. The phrase does not, of course, exclude inadvertent, coincidental, or normal exposure of the affected tissues to light.

The following examples are intended to  
35 illustrate but not to limit the invention.

- 20 -

Example 15        Prevention of Restenosis in Rabbits

Two groups of eight rabbits each were induced to an atherosclerotic state by an atherogenic diet and balloon-stretch injury of the aorta as described previously Johnson, et al. Laser Research in  
 10        Medicine (1987) 10:13-17. The experimental group received 2 mg/kg BPD-MA intravenously at the time of the injury, and one, two and three months later; the control group received no drug. The smooth muscle cell proliferation and intimal hyperplasia was  
 15        quantitated in both groups at four months by sacrificing the animals and comparing the magnitude of formation by microscopy. The results were evaluated as percentage of occlusion and thickness of the  
 20        intimal hyperplasia. Percent occlusion used 6 diameters yielding 12 points on the vessel wall, in a clock-like format. Percent occlusion was calculated by the formula

$$25 \quad 1 - \frac{\sum (\text{plaque to plaque distance})^2}{\sum (\text{wall to wall distance})^2} \times 100 = \% \text{ vessel closed.}$$

For intimal hyperplasia thickness, 12 measures were taken in a clock-like format and the average of 12  
 30        thickness measurements was computed.

On gross examination, the controls had diffuse IH throughout the aorta in seven out of eight animals. The experimental group had no IH in four out of eight animals, only one small area in three out of  
 35        eight and a mild diffuse IH in one. When evaluated by microscopy, the mean IH thickness in the control group was 190 $\mu$  and in the experimental group 21 $\mu$ ; normal media thickness was 140 $\mu$ . Lumen reduction by IH was

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27% in the controls and only 3% in the BPD-treated  
5 rabbits. Media invasion and wall calcification were  
seen in six and five of the eight controls,  
respectively, but in none of the test animals. Smooth  
muscle cells were present in IH of all animals showing  
IH, and were dense in controls and less dense in BPD  
10 treated animals. Thus, BPD-MA markedly inhibits IH in  
this model.

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CLAIMS

- 5  
1. A pharmaceutical composition for  
inhibiting the development of intimal hyperplasia  
following angioplasty, wherein said composition  
comprises an amount of green porphyrin (Gp) effective  
10 to inhibit said development when administered to a  
subject in conjunction with said angioplasty and said  
inhibition is allowed to occur in the absence of  
irradiation with light absorbed by said Gp.
- 15  
2. The pharmaceutical composition of claim  
1 wherein said Gp is administered in 2-5 doses, with  
the first said dose being administered within 6 hours  
of said angioplasty.
- 20  
3. The pharmaceutical composition of claim  
2 wherein said effective amount is 0.01-5 mg/kg per  
dose.
- 25  
4. The pharmaceutical composition of claim  
3 wherein said effective amount is 0.05-2 mg/kg per  
dose.
- 30  
5. The pharmaceutical composition of claim  
1 wherein said Gp is administered in a liposomal  
formulation.
- 35  
6. The pharmaceutical composition of claim  
1 wherein said Gp is of the formula 1-6 as shown in  
Figure 1.
7. The pharmaceutical composition of claim  
6 wherein R<sup>1</sup> and R<sup>2</sup> are carbomethoxy and carboethoxy.



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8. The pharmaceutical composition of claim  
5 6 wherein each R<sup>3</sup> is -CH<sub>2</sub>CH<sub>2</sub>COOH or a salt, amide, ester  
or acyl hydrazone thereof.

9. The pharmaceutical composition of claim  
6 wherein said Gp is of formula 3 or 4.

10 10. The pharmaceutical composition of claim  
9 wherein said Gp is selected from the group  
consisting of BPD-DA, BPD-DB, BPD-MA and BPD-MB.

15 11. The pharmaceutical composition of claim  
10 wherein said Gp is BPD-MA.

12. A pharmaceutical composition for  
inhibiting the development of intimal hyperplasia  
20 following angioplasty, wherein said composition  
comprises an amount of green porphyrin (Gp) effective  
to inhibit said development when administered to a  
subject in conjunction with said angioplasty and said  
inhibition is allowed to occur in the absence of  
25 irradiation with light absorbed by said Gp.

13. The pharmaceutical composition of claim  
12 wherein said composition is administered in 2-5  
doses, with the first said dose being administered  
30 within 6 hours of said angioplasty.

14. The pharmaceutical composition of claim  
13 wherein said effective amount is 0.01-5 mg/kg per  
dose.

35 15. The pharmaceutical composition of claim  
14 wherein said effective amount is 0.05-2 mg/kg per  
dose.

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16. The pharmaceutical composition of claim  
5 12 wherein said Gp is administered in a liposomal  
formulation.

17. The pharmaceutical composition of claim  
12 wherein Gp is of the formula 1-6 as shown in Figure  
10 1.

18. The pharmaceutical composition of claim  
17 wherein R<sup>1</sup> and R<sup>2</sup> are carbomethoxy and carboethoxy.

19. The pharmaceutical composition of claim  
15 17 wherein each R<sup>3</sup> is -CH<sub>2</sub>CH<sub>2</sub>COOH or a salt, amide,  
ester or acyl hydrazone thereof.

20. The pharmaceutical composition of claim  
20 17 wherein said Gp is of formula 3 or 4.

21. The pharmaceutical composition of claim  
20 wherein said Gp is selected from the group  
consisting of BPD-DA, BPD-DB, BPD-MA and BPD-MB.

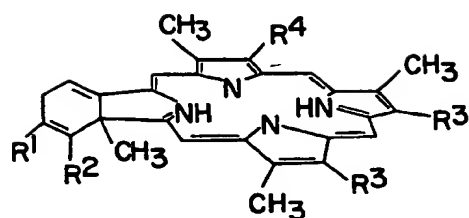
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22. The pharmaceutical composition of claim  
21 wherein said Gp is BPD-MA.

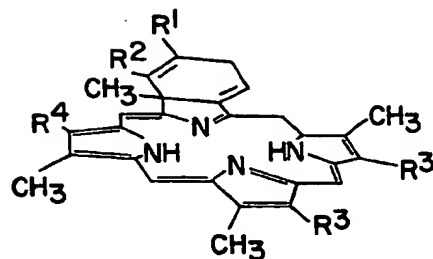
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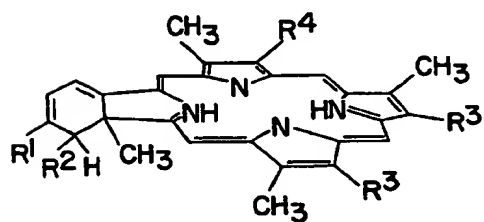
FORMULA 1



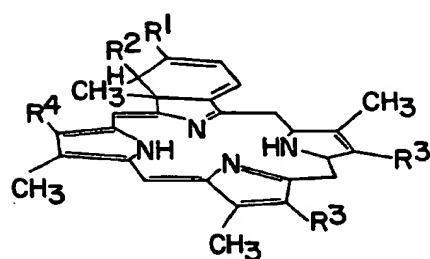
FORMULA 2



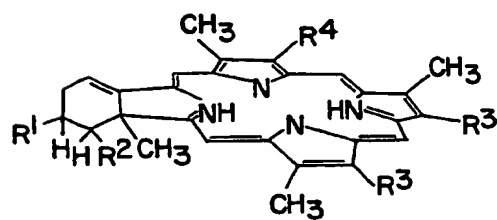
FORMULA 3



FORMULA 4



FORMULA 5



FORMULA 6

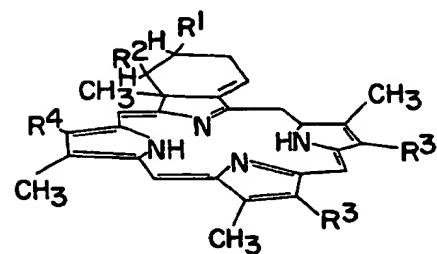


FIG. 1

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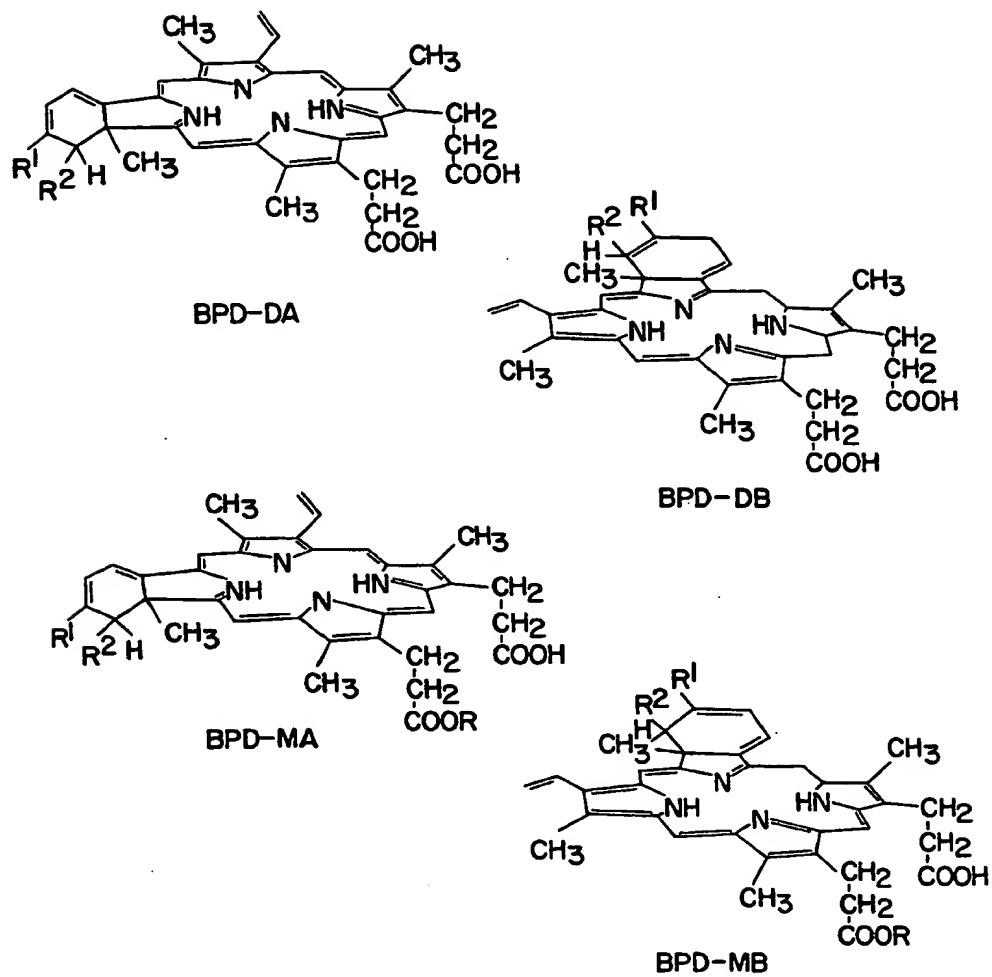


FIG. 2

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/08200

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(5) : A61K 31/40 US CL : 514/410 According to International Patent Classification (IPC) or to both national classification and IPC																				
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 514/410  Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)																				
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>																				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																		
A,P	US, A 5,314,905 (Pandey et al) 24 May 1994, see entire document.	1-22																		
A,P	US, A 5,308,861 (Aizawa et al) 03 May 1994, see entire document.	1-22																		
A,P	US, A, 5,308,608 (Dolphin et al) 03 May 1994, see entire document.	1-22																		
A,P	US, A, 5,283,255 (Levy et al) 01 February 1994, see entire document.	1-22																		
X	ATHEROSCLEROSIS, Vol. 10, No. 4, issued July 1990, Dartsch et al, "Differential Effect of Photofrin II on Growth of Human Smooth Muscle Cells from Nonatherosclerotic Arteries and Atheromatous Plaques in Vitro", pp.616-624, see entire document.	1-22																		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.																				
<table border="0"><tr><td>* Special categories of cited documents:</td><td>*T</td><td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td></tr><tr><td>*A* document defining the general state of the art which is not considered to be of particular relevance</td><td>*X*</td><td>document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td></tr><tr><td>*E* earlier document published on or after the international filing date</td><td>*Y*</td><td>document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td></tr><tr><td>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td><td>*G*</td><td>document member of the same patent family</td></tr><tr><td>*O* document referring to an oral disclosure, use, exhibition or other means</td><td></td><td></td></tr><tr><td>*P* document published prior to the international filing date but later than the priority date claimed</td><td></td><td></td></tr></table>			* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	*E* earlier document published on or after the international filing date	*Y*	document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G*	document member of the same patent family	*O* document referring to an oral disclosure, use, exhibition or other means			*P* document published prior to the international filing date but later than the priority date claimed		
* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention																		
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Date of the actual completion of the international search 14 SEPTEMBER 1994		Date of mailing of the international search report OCT 20 1994																		
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer JAMES H. REAMER Telephone No. (703) 308-1235																		

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/08200

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CIRCULATION, Vol. 85, No. 3, issued March 1992, Ortu et al, "Photodynamic Therapy of Arteries", pp.1189-96, see page 1190.	1-22
X	JOURNAL OF SURGICAL RESEARCH, Vol.53, No. 6, issued 06 December 1992, Eton et al, "Inhibition of Intimal Hyperplasia by Photodynamic Therapy Using Photofrin", pp. 358-62, see pages 360-61.	1-22